

**Effects of aquatic habitat degradation on hybridization between two species of Sunfish:**

**Bluegill (*Lepomis macrochirus*) and Green Sunfish (*Lepomis cyanellus*)**

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**Major: Forestry, Fisheries, and Wildlife; Fisheries and Aquatic Sciences**

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## Abstract

Stream habitat degradation includes factors such as as increased turbidity and excessive sedimentation of the streambed resulting from surrounding urban land use and development. It is hypothesized that these two physical characteristics of streams have the greatest effect on hybridization of closely related fishes, leading to decreased biodiversity. The objective of this research was to determine if there is a correlation between substrate sedimentation, turbidity, and Hybrid Sunfish abundances. Hybridization between Bluegill (*Lepomis macrochirus*) and Green Sunfish (*Lepomis cyanellus*; an indicator of poor water quality), referred to as Hybrid Sunfish, was investigated. Additionally, species composition in degraded systems was investigated to determine if there is a relationship between habitat quality and Bluegill abundances. Four sections of the Olentangy River, near the Ohio State University, specifically within a reach of the stream that has recently been restored, were sampled a total of three times each using standard fish collection techniques. This yielded the sunfish species composition for each site. The Ohio EPA Qualitative Habitat Evaluation Index (QHEI) was used to determine quality scores for total habitat quality, and various habitat characteristics, within each of the four sections of the Olentangy River. Our data illustrates a negative correlation between the relative abundance of Hybrid Sunfish and total QHEI scores, substrate quality, quality and amount of available instream cover, and quality of pool habitat, suggesting that there are more hybrids in lower quality habitats. Furthermore, a positive correlation between the relative abundance of Bluegill and total QHEI scores, substrate, instream cover, and pool quality was found. These findings contribute to our current understanding of how degraded aquatic systems may affect biodiversity. They also support existing tolerance classifications for both Bluegill and Green Sunfish.

## **Introduction**

Human impacts on aquatic habitats are evident worldwide. These impacts include channelization of streams, destruction of crucial riparian buffer zones, water pollution, flow modification, and destruction and degradation of habitat (Dudgeon et al., 2006). This is not an issue of recent concern; because humans have relied so heavily on waterways for transport of goods and people, almost all major cities have been constructed near waterways. However, the increase in dam construction in the Tennessee River Valley by the Tennessee Valley Association (TVA), as well as in other parts of the United States, after the Great Depression is a perfect example of how increased development has harmed streams (Kitchens, 2014). The TVA increased dam construction in the 1930's to generate jobs and provide an inexpensive form of energy to the Southeastern United States (Kitchens, 2014). Human-induced habitat degradation, specifically resulting from urban development and dam construction, transforms the look and function of streams and creates environments not suitable for certain species to inhabit (Perkin et al., 2015).

### *Habitat Degradation*

Aquatic habitat degradation is best characterized by Walsh et al.'s (2005) definition of the urban stream syndrome. Symptoms of the urban stream syndrome include: a flashy hydrograph, elevated concentrations of both nutrients and contaminants, altered channel morphology, reduced biodiversity, and an increase in tolerant species abundances (Walsh et al. 2005; Paul and Meyer 2001; Meyer et al. 2005). Overall stream habitat quality is commonly inversely related to the amount of urbanization within the watershed (Wang et al., 2001; Vietz et al., 2014). Watershed urbanization results in behavioral, chemical, and geomorphological

changes to the stream (Wang et al., 2001; Clements et al., 2000; Vietz et al., 2014). These changes are mainly due to the increase in impervious surface area in urban areas that prevents precipitation from infiltrating the soil and slowly make its way to the stream (Walsh et al., 2005; Vietz et al., 2014). Rather, precipitation is diverted directly to stream channels through sewer systems or by over-the-surface flow. This causes an increase in runoff volume and an increase in magnitude and frequency of flooding, creating larger flow events with faster increasing and decreasing hydrographs (Vietz et al., 2014; Walsh et al., 2005). Increases in flooding increase stream bank erosion, which destroys pool habitat and instream cover – consisting of vegetation, debris, and boulders in, and overhanging, the stream – and leads to streambed scour and sediment deposition (Wang et al., 2001; Walters et al., 2003). Additionally, urban runoff is typically polluted with oils and chemicals from automobiles, as well as sediments from roadways, or partially treated wastewater. These pollutants further decrease water quality and can cause declines in abundances of pollution-intolerant aquatic organisms by increasing nutrient loads and reducing dissolved oxygen levels downstream of inputs (Katz and Gaufin, 1953; Walters et al., 2003; Walsh et al., 2005; O’Driscoll et al., 2010). Urban streams are also generally found with higher concentrations of heavy metals (mercury and cobalt), hydrocarbons, and organics which can adversely affect the health of aquatic communities (O’Driscoll et al., 2010). For example, more sensitive (i.e. intolerant) cold-water fish species, like Brook Trout (*Salvelinus fontinalis*), Brown Trout (*Salmo trutta*), Rainbow Trout (*Onchorhynchus mykiss*) and Sculpin (*Cottus* spp.), were not found in streams with even a low percentage of impervious building material (8-10%) (Standfield and Kilgour, 2012). In contrast, similar streams in the same area that were less urbanized (i.e. lower percentage of impervious material) were able to support these species.

The total area, or percentage area, of impervious surface present in a watershed is an important factor that affects stream health (Wang et al., 2001; O'Driscoll et al., 2010; Stanfield and Kilgour, 2012.). However, the amount of impervious surface area is not the only watershed characteristic that affects stream habitat quality. The width of forested riparian buffers is positively related to fish and invertebrate biotic integrity, a measure of an organism's function in comparison to non-human altered systems (Wang et al., 2001; O'Driscoll et al., 2010). This is because riparian buffers serve as barriers that help filter excess nutrients and sediment from runoff before it enters the stream (Lowrance et al., 1984; Daniels and Gilliam, 1996; O'Driscoll et al., 2010). Excess nutrients, chemicals, and sediments can increase algal concentrations, increase instream temperatures, induce chemical stress in aquatic organisms, and deposit sediment onto sensitive benthic habitats. These alterations may then influence species composition by negatively impacting sensitive vertebrate and invertebrate species, thus potentially decreasing their overall abundances (Walters et al., 2003; Walsh et al. 2005). Increased sedimentation can obliterate sensitive benthic habitats where invertebrates thrive, and limit the heterogeneity of benthic habitat.

Lenat and Crawford (1994) and others have associated increases in urbanization with increased erosion, sedimentation, and the loss of instream habitat (Schueler, 1994; Arnold and Gibbons, 1996; Booth and Jackson, 1997; May et al., 1997). Because of increased frequency and magnitude of hydrologic events, channel complexity and instream habitat heterogeneity are commonly observed in degraded urban streams (Walsh et al., 2005). Additionally, species compositions in urban streams are typically made up of a less diverse and more tolerant group of species (Walsh et al., 2005). Eitzman and Paukert (2010) found that in the Kansas River, a system with a homogenized assemblage of species longitudinally, differences in habitat resulted

in the variation in fish community composition. This illustrates that as habitat complexity decreases, the ability to support complex fish communities also decreases. A loss of instream habitat translates into a loss of suitable habitat for all species to thrive. Intolerant species – those classified as unable to tolerate poor water quality or low habitat complexity – are more likely to be found in river reaches that are less urbanized and impacted by channelization (Eitzman and Paukert, 2010). Decreases in availability of habitat tends to increase competition between organisms, thus most likely leaving only the tolerant and generalist species, those that can utilize un-preferential habitats and food sources, to succeed. Eitzman and Paukert (2010) found a higher abundance of generalist omnivores in the channelized portions of the Kansas river. In the un-channelized portions, they found higher abundances of insectivorous species. As a result of increased competition for food sources in degraded systems, the less tolerant insectivorous species could not thrive.

Agricultural land use in watersheds also has habitat degrading qualities (Allan, 2004). Soil erosion and excessive nutrient inputs have degrading effects on aquatic ecosystems (Allan, 2004; Ehlman et al. 2015). As a result of soil erosion and excess nutrients, increases in turbidity and harmful algal blooms are common in these affected ecosystems (Quinn et al., 1997; Allan, 2004). Turbidity characterizes the clarity of water as a result of the amount of suspended particle matter in the form of sediment or algae. Turbidity, both algal and sedimentary, absorbs and reflects different wavelengths of light that penetrate the water column. Chronic turbidity can disrupt visual cues and lead to dull mating coloration in fish (Ehlman et al. 2015). Excess nutrients are also associated with high sedimentary turbidity. When soil particles are washed into the stream, they lose their attached ions, specifically nitrates and phosphates, which also become suspended. This allows harmful algal blooms to arise because of the increase in available

nutrients. Therefore, harmful algal blooms can occur when a body of water is polluted with excess nutrients (Quinn et al., 1997). These nutrients also allow for the abundant growth of various types of algae, which further increases turbidity. Upon the death of algal blooms, microbial decomposition uses up massive amounts of dissolved oxygen creating dead zones in affected waters where there is little to no oxygen for aquatic organisms to utilize (Anderson et al., 2002). This creates a positive feedback loop, further decreasing habitat quality.

The physical changes to streams linked to urbanization and agriculture can have a direct effect on fish diversity and abundance (Karr, 1981; Walters et al., 2003). A loss of instream cover, and an increase in sedimentation, have negative effects on the amount of overall suitable habitat. As sediment and habitats become homogenized, fish lose valuable refuge, foraging, and spawning areas. A loss of preferred habitat in an ecosystem condenses organisms and drives increased resource competition (Werner and Hall, 1977). This is especially true for species such as Bluegill and Green Sunfish that reproduce and forage in similar environments (Werner and Hall, 1977).

### *Bluegill and Green Sunfish*

Bluegill and Green Sunfish are commonly found together in streams and lakes in central North America and are only physically separated by their preferred habitats within those streams and lakes (Werner and Hall, 1977). Both Bluegill and Green Sunfish belong to the family Centrarchidae. These two species are found in lakes, ponds, rivers, creeks, and swamps, and have relatively similar habitat and water quality preferences (Jester et al., 1992). However, Green Sunfish exhibit higher tolerance to habitat and water quality disturbances than Bluegill. Green Sunfish show increased abundance in waters with recent degradation and become a dominant

species in disturbed systems (Karr, 1981; Karr et al., 1986). Because of this, the presence and abundance of Green Sunfish is used as a metric in the Index of Biotic Integrity (IBI) (Jester et al., 1992; Karr, 1981; Karr et al., 1986). The IBI utilizes characteristics of stream ecosystems such as fish species composition, percentages of tolerant and intolerant fish species, and percentage of diseased fish, to assess the effect of human influence on stream health (Karr, 1981; Karr et al., 1986). Each characteristic is defined as a metric with associated scoring breakpoints, low scores are attributed to streams with high human disturbance, and vice-versa. The proportion of individuals of Green Sunfish is one of the metrics, and a low score is given to this metric when proportions of Green Sunfish are high (Karr, 1981; Karr et al., 1986).

In lakes and ponds, Bluegill generally prefer the deeper water column or the littoral zone between 1-6 m depth, while Green Sunfish prefer shallow areas, typically <1 m deep, near aquatic vegetation (Werner and Hall, 1977). A study by Werner and Hall (1977) determined that when Green Sunfish and Bluegill inhabit the same body of water, Bluegill forage in open water on zooplankton and small aquatic invertebrates, and Green Sunfish forage on organisms associated with aquatic vegetation. However, when the two species are allopatric (not present together in the same lake or pond), Bluegill feed on the vegetation-dwelling prey, not zooplankton in open water (Werner and Hall, 1977). Bluegill may shift from their preferred foraging habitat to a secondary foraging habitat in the presence of Green Sunfish – a possible result of resource competition between the two species (Robinson and Wilson, 1994).

Green Sunfish are voracious, sit-and-wait predators with large mouths, and moderately fusiform bodies that allow them to feed more efficiently on larger vegetation-dwelling prey (Werner and Hall, 1977). Their body form allows for fast, forward, ambush-like movements. Alternatively, Bluegill have smaller mouths, laterally compressed bodies, and tend to school



(outside of the mating season). These traits make Bluegill more efficient at preying upon smaller prey in higher abundances, such as zooplankton (Werner and Hall, 1977). Their compressed bodies allow for increased maneuverability that is needed to forage for small prey. Therefore, Bluegill cannot compete with Green Sunfish when sympatric (living in the same habitat), and must shift to another habitat or resource. However, in an urbanized body of water, the habitat Bluegill would shift to, in this case, may not be present (Werner and Hall, 1977; Wang et al., 2001). This may inevitably lead to a decline in abundance of Bluegill, which may make finding potential mates difficult during the spawning season.

There are 37 species in the Family Centrarchidae, making it the second largest fish family native to North America. In centrarchids, the male builds a nest, courts, attracts a female to the nest, and spawns with the female (Cooke et al., 2008). The male nest builder is also the sole provider of protection for the offspring until the juveniles become independent (Cooke et al., 2008). Because of their similar reproductive patterns and strategies, hybridization between centrarchid species has been found to be fairly common (Breder, 1936; Cooke et al., 2008).

In general, the spawning season for centrarchids begins as the water temperature reaches 10 °C in the spring, at which time males begin to build nests in shallow water (Breder, 1936). Many factors play a role in the overall location and structure of the nests, including water depth, nearby objects, size of the male, and proximity to other nests (Breder, 1936; Avila, 1976). Avila (1976) found that in increased nesting densities, where available nesting substrate is limited, nesting males exhibited increased aggression towards one another. Urbanization may influence these factors through both chemical and physical changes to the stream, especially through increasing sediment deposition within a stream. In general, the male centrarchid builds a nest that has a diameter roughly twice the length of the male, although the density of neighboring nests

affects the overall size of the nest (Breder, 1936; Avila, 1976). By fanning away any sand with its tail and repositioning gravel with its mouth, the male creates a circular depression in the stream or lakebed for the female to lay her eggs in (Breder, 1936; Jennings and Philipp, 2002). Because the male can only utilize his tail and mouth to create the nest, sunfish are typically not observed spawning in overly sandy or silt-covered areas (Avila, 1976). Males that attempt to build a nest in silt merely end up creating a silt cloud in the water that resettles over the nest, smothering any eggs present. Centrarchids, therefore, prefer gravel and other materials that can be easily cleared (Avila, 1976). Nest building territory is significantly reduced in urbanized streams severely affected by fine sediment deposition. Since sunfish do not spawn in areas with fine sediment and urbanized streambeds are covered with fine sediment, the abundance of suitable nesting substrate is significantly decreased (Avila, 1976; Walters et al., 2003). Therefore, nests of multiple sunfish species are densely packed into the streambed, potentially facilitating hybridization, either through increasing the chance of females mating with males of the wrong species or by allowing cuckolding or “sneaker” male centrarchids easier access to nearby nests.

When spawning, males and females of most centrarchid species can be differentiated based on the males’ distinct patterning and courtship rituals (Breder, 1936). If water clarity is compromised due to sedimentary or algal turbidity, certain wavelengths of light are scattered and absorbed, altering the perception of spawning colors and increasing the difficulty for fish to detect contrasts in color (Lythgoe, 1984). Therefore, differentiating males of different species may be difficult for females in turbid waters and lead to hybridization if females choose to lay their eggs in the nest of a male of the wrong species. This process has been identified in a genus

of Lake Victoria cichlid fish, *Pundamilia*, in which closely related species interbreed in turbid waters, but not in clear waters (Maan et al, 2010).

A female centrarchid can either deposit all of her eggs at once, or at multiple times in different nests. However, upon deposition her eggs are susceptible to fertilization by whatever sperm they come into contact with (DeWoody et al., 1998). This is another time where hybrid fertilization can occur via sperm deposition by a cuckolding or “sneaker” male (Jennings and Philipp, 2002). “Sneakers” are generally smaller males that have reached sexual maturity much earlier in life than the older nest-making males (Garner and Neff, 2013). The smaller “sneaker” males do not express typical male coloration, build nests, or court females, instead they devote their entire reproductive effort on cuckolding (Jennings and Philipp, 2002). Cuckolding males wait for the female to lay her eggs in the nest she chose to spawn in. Upon egg deposition the “sneaker” males sneak into the nest, and attempt to fertilize her eggs before the male who built the nest can either deposit his sperm or drive the “sneaking” male away (Ughlem and Rosenqvist, 2002). Male “sneakers” eliminate the ability for a female to actively choose a mate (Garner and Neff, 2013; Jennings and Philipp, 2002), and in some cases the cuckolding males do not always effectively discriminate between centrarchid species, potentially facilitating hybridization (Garner and Neff, 2013). This is a definite possibility, especially when visibility in the stream is hindered due to increased turbidity and species-specific details of male patterning are unclear.

### *Hybridization*

Hybridization between two species of fish occurs naturally in many ecosystems, but the frequency at which hybridization naturally occurs is typically quite low (Hubbs, 1955).

However, because the family Centrarchidae is the second largest family of fish and will hybridize naturally, they are often an area of focus for hybridization research (Hubbs, 1955; Avila, 1976). Some sunfish species, such as Green Sunfish and Pumpkinseed, are so capable of hybridizing that merely placing a male of one species and a female of the other into an aquarium may initiate courting rituals (Hubbs, 1955). Shortly after introduction, the female lays her eggs and the male fertilizes them. Multiple eggs hatch and develop into mature adults whose characteristics are the intermediate of its parents' (Hubbs, 1955). Hubbs (1955) was able to reproduce the same results with many other combinations of sunfish species, including the two examined in the present study, Bluegill and Green Sunfish.

The hybrid centrarchids produced from interspecies crosses have similar behavioral traits to their parent species, but generally grow faster than their pure parental species and excel in regards to holding fins erect and the depth, brightness, and intensity of color (Hubbs, 1955). Additionally, when hybrids and pure species are fed, hybrids take the food first and show increased ability to forage for food (Hubbs, 1955; Childers & Bennett, 1961). Hybrid Sunfish males also construct, fan, and guard their nests with increased aggressiveness for a longer period of time (Hubbs, 1955). The interbreeding of a male Bluegill and a female Green Sunfish typically yields roughly 80% fertile male hybrid offspring (Winkelman and Sager, 2002). Due to the male dominated offspring, their potential for overpopulation is low. Their aggressive tendencies also cause them to fight harder and longer once hooked by a fisherman (Winkelman and Sager, 2002). Both of these reasons make Hybrid Sunfish preferential for stocking.

The factors that influence the occurrence of hybridization between Bluegill and Green Sunfish, as well as other centrarchids, are not completely understood. However, there is a general consensus that degraded habitats play a major role in influencing hybridization to occur in

nature. Hybrids appear to be more common in environments where there is an extremely high abundance of aquatic vegetation and in waters with high turbidity (Hubbs, 1955). This is thought to be the result of the pure species' inability to differentiate the male and female of the other species when searching for a mate. Due to the scattering and absorption of light wavelengths by increased turbidity and the obstruction of the fish's field of view by the vegetation, males and females of different species may be unable to see the proper courtship displays and spawning colorations and consequently interbreed (Hubbs, 1955; Ehlman et al. 2015).

The decrease in visibility associated with increased turbidity caused by bank erosion and sediment load in a stream hinders a fish's ability to see courtship displays (Hubbs, 1955;). This hypothesis, made by Hubbs (1955), serves as a foundation in the search for factors impacting hybridization between these two centrarchid species. Additionally, Hubbs (1955) identified the shortage of conspecific mates as a driver for hybridization. Cuckolding males have also been hypothesized to be drivers facilitating hybridization (Garner and Neff, 2013). Cuckolding males have to act quickly in order to be successful, so they can deposit their sperm before being driven off by the male owner of the nest. However, acting quickly means there is room for error when selecting the nest to fertilize to ensure conspecific breeding is occurring (Garner and Neff, 2013). This mechanism of hybridization is beneficial to the cuckolding male, because it involves no parental care. However, it is costly to the female who laid the eggs and the male that provides the parental care (Jennings and Philipp, 2002). Garner and Neff (2013) determined that cuckolding Bluegills are responsible for the asymmetrical hybridization between Bluegill and Pumpkinseed. However, no evidence of Pumpkinseed spawning in Bluegill nests was found, but cuckolding Bluegill frequently fertilized Pumpkinseed nests (Garner and Neff, 2013). Garner and Neff (2013) hypothesized that this could have resulted from a barrier to effective mate recognition.

Jennings and Philipp (2002) also looked to cuckolding males for an explanation of hybridization. In areas where nests are densely packed and in close proximity to one another, neighboring males may sneak into another's nest when there is a shortage of conspecific mates (Jennings and Philipp, 2002).

### *Objectives, Hypotheses, and Predictions*

The objective of this study was to determine the possible effects that habitat degradation has on hybridization between Bluegill and Green Sunfish. We hypothesized that habitat degradation would play a key role in influencing hybridization, because of the combined effects of turbidity, loss of foraging habitat, and loss of spawning substrate; which are characteristic of degraded habitats. Therefore, we predicted we would find higher Hybrid Sunfish abundances in more degraded habitats. We also sought to determine what effect habitat quality has on Bluegill abundances, hypothesizing that habitat quality also plays a key role in determining Bluegill abundances. Bluegill are somewhat-sensitive to pollution and degradation; therefore, it is thought that habitat quality will affect their overall abundances. We predicted we would find higher Bluegill abundances in less degraded habitats.

### **Methods**

To test if habitat degradation influences hybridization between Bluegill and Green Sunfish, we sampled fish populations, tested water quality, and measured habitat quality – as well as various habitat characteristics – from four different sections of the Olentangy River. The Olentangy River flows downstream from Galion, Ohio to its confluence with the Scioto River in Columbus, Ohio, a total stream distance of 149 miles (Ohio EPA, 2007) (Fig. 1). The Olentangy

River watershed encompasses 1406 km<sup>2</sup> in Central Ohio and is dominated by agricultural usage. However, the watershed in Franklin County, where our sampling sections were, is entirely of urban land use (Ohio EPA, 2007). Additionally, the area of the watershed upstream of the urban usage is currently experiencing a land-use transition from agricultural to urban. Overall, the land use in the Olentangy watershed is 56% cropland, 14% urban, 14% forested, 13% pasture (Ohio EPA, 2007); however, as stated previously, a large proportion of the watershed is becoming increasingly urbanized.

We delineated four, 300 m long sections of the Olentangy River near The Ohio State University campus. The placement of the sampling sections allowed for a gradient of habitat conditions to be assessed, because the sites shifted from a less-urban to more-urban immediate surrounding landscape. These 300m long sections were a minimum of 300 m apart from one another. Gatz and Adams (1994) found that Bluegill, along with Redbreast Sunfish (*Lepomis auritus*), Rock Bass (*Ambloplites rupestris*), Largemouth Bass (*Micropterus salmoides*), and Warmouth (*Lepomis gulosus*), were generally sedentary and typically migrated less than 100m upstream and downstream over the course of their three-year study. Therefore, the 300m separation between sections provided some definition between the four fish assemblages, i.e. one semi-distinct assemblage per section (Fig. 2, Fig. 3). The first sampling section was directly downstream of the low-head dam on the Olentangy River near the OSU Wetlands Research Park (Fig. 2; Fig. 3) and Dodridge Road. The remaining three sections were downstream throughout the restored section of the Olentangy River, with the final section located directly downstream of the location of the removed low-head dam on 5<sup>th</sup> Avenue.

### *Measuring Fish-Species Abundances*

Centrarchid species abundances were measured at each of the four sites, passing through each site three times with seine nets every sampling day. We sampled each site three different times for a total of 12 sampling days (i.e. 4 sites x 3 sampling visits each). At the end of the sampling visit, we weighed (g) and measured the standard and total lengths (cm) of each fish caught. Photos of individual fish were also taken in the field after netting the fish. This allowed for laboratory identification when we were unable to determine if an individual was a Hybrid Sunfish, Bluegill, or Green Sunfish. In order to determine the relative abundance of each species caught, we calculated the proportion of each (i.e. species abundance divided by total centrarchid abundance). For this study, we were only interested in the proportions of each sunfish species relative to the total number of sunfish caught, as opposed to their abundances in relation to all species caught.

### *Measuring Water Quality*

Water quality parameters (dissolved oxygen (mg/L), temperature (°C), and conductivity (µs) were measured at each site using a YSI Pro2030 multimeter to coincide with fish sampling. Three turbidity samples were taken at the time fish sampling was completed at each site, measured in the lab using a LaMotte 2020e portable turbidity meter (NTU), and then averaged. A total of 36 turbidity samples were collected throughout the study (i.e. 3 turbidity samples x 3 sampling days x 4 sites). Dissolved oxygen, temperature, and conductivity were also measured once per sampling day, for a total of 12 water quality measurements taken throughout the study.



### *Measuring and Quantifying Habitat Quality*

The Qualitative Habitat Evaluation Index (QHEI) was used to measure and determine habitat quality scores. The QHEI is a method of quantifying habitat quality within a stream (Rankin, 1989). It requires measurement of seven habitat characteristics that are used to produce a score for each metric along with a total habitat quality score: substrate, instream cover, channel morphology, bank erosion and riparian zone, pool quality, riffle quality, and gradient (Table 1). We completed the QHEI once for each section of the stream sampled, resulting in four total QHEI scores.

### *Analyses*

To test the prediction that habitat degradation and the abundance of Hybrid Sunfish are positively correlated, we first calculated the proportion of Hybrid Sunfish in relation to the total number of sunfish caught. This gave us the relative abundance of sunfish that was found at each site. Relative abundances were also calculated for Bluegill and Green Sunfish. The proportion of hybrid individuals was then compared to turbidity, total habitat quality scores, and all of the habitat variables that may influence hybridization as described above. This comparison was also done for Bluegill and Green Sunfish in order to determine if a correlation existed for these species as well. A multiple linear regression, performed using Microsoft Excel, was then used to determine if there was a relationship between the tested habitat variables and the proportion of hybrid individuals, Bluegill, and Green Sunfish. Analysis of variance (ANOVA) tests, also performed in Excel, were utilized to determine if turbidity and dissolved oxygen differed between sites. After initial analysis of the QHEI data was completed, individual QHEI metrics were investigated to determine which may have had the greatest influence on

hybridization. The coefficients of variation were calculated for each QHEI metric in order to determine which metrics varied the most between all sites.

## **Results**

### *Fish Sampling Results*

Four sunfish species were caught and included in the analysis; Bluegill, Green Sunfish, Orangespotted Sunfish (*Lepomis humilis*), and Hybrid Sunfish. Due to the fact that Bluegill and Green Sunfish can also hybridize with Orangespotted Sunfish, we included this species to ensure all sunfish were represented. At site 1, at Dodridge Rd. (Fig. 2, 3), the relative abundance of Bluegill was found to be the highest of all of the sites. In contrast, at site 3, the Ohio Stadium, the relative abundance of Bluegill was the lowest, no Bluegill were caught. Therefore, at site 3, only two sunfish species were caught, while four species were caught at site 2. The relative abundances of Green Sunfish and Hybrid Sunfish were also lowest at site 1 (Table 2). In contrast, Site 4, at 5<sup>th</sup> Ave., had the highest relative abundance of Hybrid and Green Sunfish (Table 2).

### *Water Quality Results*

Site 4 had the highest mean turbidity throughout the study period, while site 3 had the lowest mean turbidity (Table 3; Fig. 4). Site 4 was also found to have the highest concentration of dissolved oxygen (Table 3; Fig. 5). In contrast, site 3 was found to have the lowest concentration of dissolved oxygen. Single factor ANOVA tests were completed with a 95% confidence level on average turbidity (NTU) and average dissolved oxygen concentration (mg/L), comparing the averages between all sites and sampling days. However, neither turbidity

or dissolved oxygen concentration showed statistically significant differences across sites. The p-values of average turbidity and average dissolved oxygen were 0.666 and 0.808, respectively. Indicating there was no statistical difference in any turbidity and dissolved oxygen concentration measurements taken between sites.

### *Habitat Quality Results*

The total QHEI scores followed a general decreasing trend from site 1 to site 4 (Fig. 6). Site 1 was found to have the highest total QHEI score, while site 3 was found to have the lowest. Therefore, site 1 exhibited the highest quality habitat out of all of the study sites. With a total habitat quality score of 53, site 1 ranked as “fair” (scores of 46-59) by QHEI standards. The other three sites were ranked as “poor” (scores of 30-45).

Overall, there was a positive relationship between habitat quality and the relative abundance of Bluegill (Fig. 7). As habitat quality increased, the relative abundance of Bluegill increased ( $r^2 = 0.951$ ,  $p = 0.025$ ). In contrast, there was a negative relationship between habitat quality and the relative abundance of Green Sunfish and Hybrid Sunfish (Fig. 7). As habitat quality decreased, the relative abundance of Hybrid Sunfish and Green Sunfish increased, though these relationships were not significant (Green Sunfish  $r^2 = 0.820$ ,  $p = 0.165$ , and Hybrid Sunfish  $r^2 = 0.698$ ,  $p = 0.095$ ).

### *Coefficient of Variation Results*

Most of the scores of the individual metrics within the QHEI varied between all sites; however, gradient and channel morphology differed the least with coefficients of variation of 0

and 0.077, respectively (Table 4). The coefficient of variation was greatest for instream cover and substrate (Table 4). Oppositely, the coefficient of variation was near zero for channel morphology and zero for gradient; meaning there was hardly any difference in these metrics between sites (Table 4). Based on the coefficients of variation for each metric, we excluded the metrics that contributed very little to the differences in habitat quality between sites when completing statistical analyses, these metrics being morphology and gradient. In doing this, we were trying to identify which metrics were required by each species for their relative abundances to stay relatively constant. We called the sum of the scores of these essential habitat metrics “critical habitat quality score”, which represented the critical habitat quality needed to support abundances of each species. Critical habitat, in terms of the Endangered Species Act, represents the parameters of habitat that are essential to the survival and recovery of a species (Camaclang et al., 2014). The species investigated in this study are not endangered; however, we are using the phrase “critical habitat quality” to describe the quality of habitat that is needed to prevent hybridization from occurring.

A simple regression test with a 95% confidence level was completed using Excel in order to produce an F-value representing statistical significance. In order to identify which metrics comprised the critical habitat of each site, the coefficients of variation were compared to one another. Habitat metrics were eliminated from the critical habitat quality score one at a time, eliminating those with the smallest coefficients of variation each time, until only the metric with the greatest coefficient of variation remained plotted against the relative abundances of each species. After each elimination, the regression test was repeated in order to test the significance of each relationship. The purpose of this sequential elimination of variables was to determine if

there was a combination of habitat variables that produced a statistical correlation between critical habitat quality and the abundances of Bluegill, Green Sunfish, and Hybrid Sunfish.

The scores of substrate, instream cover, bank erosion and riparian zone, pool quality, and riffle quality were first combined to produce the initial critical habitat quality score and was then plotted against the relative abundances of each species and hybrid sunfish (Fig. 8). This was done to test the significance of the relationship between these habitat variables and the relative abundances of each species and hybrid sunfish. A regression test with 95% confidence level was then completed. Critical habitat quality compared to relative abundance of Bluegill produced an p-value of 0.0229, indicating statistical significance. Critical habitat quality compared to relative abundance of Hybrid Sunfish produced an p-value of 0.0777, indicating no statistical significance. However, this relationship does approach significance, and has an  $r^2$ -value of 0.851, suggesting that these variables are correlated (Fig. 8). Additionally, critical habitat quality compared to relative abundance of Green Sunfish produced an p-value of 0.191, indicating no statistical significance. But again, with an  $r^2$ -value of 0.654, a correlation between these variables exists (Fig. 8).

The first habitat variable to be eliminated was bank erosion and riparian width, with a coefficient of variation of 0.253. The remaining variables; substrate, instream cover, pool quality, and riffle quality, were totaled to produce another critical habitat quality score which was plotted against each species' abundance (Fig. 9). The p-values produced from this regression were 0.0203 for Bluegill, 0.0972 for Hybrid Sunfish, and 0.162 for Green Sunfish. Indicating again that the relationship was significant for Bluegill, but not for Hybrid and Green Sunfish.

Pool quality, with a coefficient of variation of 0.270, was eliminated next. The remaining variables; substrate, instream cover, and riffle quality, were totaled to produce another critical

habitat quality score which was again plotted against each species' abundance (Fig. 10). The p-values produced from this regression were 0.0199 for Bluegill, 0.0785 for Hybrid Sunfish, and 0.231 for Green Sunfish. Indicating again that the relationship was significant for Bluegill, but not for Hybrid and Green Sunfish.

Riffle quality, with a coefficient of variation of 0.283, was eliminated next. The remaining variables; substrate and instream cover, were totaled to produce yet another critical habitat quality score which was again plotted against each species' abundance (Fig. 11). The p-values produced from this regression were 0.0102 for Bluegill, 0.108 for Hybrid Sunfish, and 0.154 for Green Sunfish. Indicating again that the relationship was significant for Bluegill, but not for Hybrid and Green Sunfish.

Substrate was the final habitat variable to be eliminated, with a coefficient of variation of 0.309, leaving just instream cover to be plotted against each species' and Hybrid Sunfish abundances (Fig. 12). The p-values produced from this regression were 0.0101 for Bluegill, 0.104 for Hybrid Sunfish, and 0.162 for Green Sunfish. Indicating again that the relationship was significant for Bluegill, but not for Hybrid and Green Sunfish.

Although, the relationship between all of the critical habitat quality scores and Hybrid Sunfish or Green Sunfish was never statistically significant, the  $r^2$ -values of each relationship were all above 0.5 and deemed correlative (Fig. 8-12). The relationship between Bluegill abundances and critical habitat quality score was found to be positively correlated no matter the combination of variables included in the critical habitat quality score (Fig. 8-12).

## **Discussion**

Overall, we found that the relative abundance of Bluegill was positively correlated with the quality of their habitat (Fig. 7). In contrast, we found the opposite relationship for Green Sunfish and Hybrid Sunfish, with higher relative abundances in lower quality habitat, though these correlations were not significant (Fig. 7). We tested the correlation between turbidity and the relative abundances of Hybrid Sunfish, Bluegill, and Green Sunfish; however, we found that turbidity between sites was not statistically different. Therefore, we were unable to complete the correlation analysis. Below, we discuss our findings pertaining to relative species abundance and habitat quality.

#### *Relative fish abundance*

Our findings suggest lower centrarchid diversity in degraded aquatic systems. Dudgeon et al. (2006) found that biodiversity across taxa is reduced in degraded stream ecosystems. Water pollution, flow modification, and degradation of habitats are some of the main threats of aquatic biodiversity (Dudgeon et al., 2006). As other another example, Lenat and Crawford (1994) found significant declines in species richness (translates to biodiversity) in the urban basins in North Carolina when compared to the forested basins they studied. These assemblages were also dominated by omnivorous and tolerant species (Lenat and Crawford, 1994). In our study, the site with the lowest habitat quality and highest level of degradation (site 3) exhibited the lowest level of biodiversity: Only two of the three sunfish species of initial interest were found at the study site with the lowest habitat quality score. This same site exhibited the highest level of sedimentation and siltation of benthic habitats. Additionally, we found the lowest habitat heterogeneity and availability of valuable instream cover at site 3. In contrast, all three species (Bluegill, Hybrid Sunfish, and Green Sunfish), were found at the sites with the lowest level of

degradation. The highest quality site (site 1) exhibited high biodiversity, preferential spawning substrate, and plentiful instream cover. Therefore, our findings suggest a negative relationship between habitat degradation and overall biodiversity. However, because of our limited habitat quality and fish assemblage data, we could not find any major differences. Biodiversity in this context only includes the comparison of the relative abundances of three species. Many more species were caught, but were not recorded due to the constraints of our study. In order to investigate the relationship between aquatic biodiversity and habitat degradation, extensive habitat quality measuring and fish sampling needs to be completed.

Jennings and Philipp (2002) and Garner and Neff (2013) hypothesized that cuckolding males could be driving hybridization. Interspecific intrusions of nests were found to occur when sunfish colonies containing more than one species, spawned in nests adjacent to one another (Jennings and Philipp, 2002). This nest intrusion by males was hypothesized to induce hybridization. Alternately, Werner and Hall (1977) found the increase in foraging and refuge competition between species could lead to decreases in the abundances of Bluegill. Given the scope of our design, we were unable to test if these processes contributed to the Hybrid Sunfish abundances we observed. Although we did find higher relative Green Sunfish abundances than Bluegill in the most degraded sites, we were unable to determine why this pattern was observed. We believe that processes such as cuckolding males and increased competition and their effects on hybridization would be more effectively tested through experimental design. Our general population study was not designed to isolate these factors as potential drivers. However, this could be potentially evaluated by rearing Bluegill and Green Sunfish in a simulated degraded environment, and observing their behaviors during foraging, nesting, and predation situations.



This would allow us to isolate habitat quality from competition factors, so that we could determine which stressor influences hybridization the most.

### *Fish Abundance and Water Quality*

Turbidity has been found to play a large role in influencing hybridization between guppies (*Poecilia reticulata*) (Ehlman et al., 2015). Hubbs (1955) found early on that the occurrence of hybridization is high in habitats with high turbidity. However, it was not clear as to why this relationship was found. Ehlman et al. (2015) found that visual cues between guppies are dulled and scattered in high turbidities. The range at which visual stimuli can be detected is also reduced under high turbidities (Ehlman et al., 2015). This affect is suggested to be similar to all fish species as well. Therefore, increased turbidity could inhibit the ability of female Bluegill and Green Sunfish to differentiate between conspecific and heterospecific mates while searching for viable males to mate with. To test if this was a factor in our study, we tested for a correlation between turbidity and the occurrence of hybrid individuals in our study sites within the Olentangy River. However, turbidity was found to not be significantly different between sites (Fig. 4). Therefore, we were unable to determine if turbidity had any influence on the relative abundances of Hybrid Sunfish, Bluegill, or Green Sunfish. Changes in heavy metal concentrations and pH have also been seen to influence the olfactory ability of fish, potentially negatively impacting the ability for individuals to accurately detect various hormones – such as those given off during spawning (Scott and Sloman, 2004). We did not test these variables; however, we did test for a correlation between the concentration of dissolved oxygen and the occurrence of hybrid individuals. Although, we have not found any support for dissolved oxygen influencing hybridization, it could play an overall role in inducing stress on Bluegill. Hypoxic

conditions, categorized by low dissolved oxygen concentrations, can cause metabolic stress in fish and other aquatic organisms which may lead to death (Cooper et al., 2002). This stress could potentially drive the decrease in the more sensitive-Bluegill abundances as habitat quality and, presumably water quality, decrease. However, the concentration of dissolved oxygen was found to not be significantly different between sites as well and was relatively high (Fig. 5). Our findings of non-significant differences in turbidities and dissolved oxygen concentrations were mainly due to the fact that this study was only conducted over the course of one season and having a very low sample size. Due to the fact all of the sites were within the same river and over a total distance of about two kilometers, it is possible that turbidity and dissolved oxygen concentration did not differ across sites because the water flows through site 1 downstream to site 4. To fully investigate this relationship, we would need many more samples over an entire year or more. This would allow us to factor in weather events and seasonal turbidity cycles, and determine an accurate average turbidity for each site. The United States Geological Survey (USGS) does monitor real-time and daily turbidity data in the Olentangy River. However, none of the USGS monitoring sites along the Olentangy River coincide with any of our study sites. If these monitoring sites did coincide with our study sites, we could substitute this data and use it for fish abundance and water quality analyses.

### *Fish Abundance and Habitat Quality*

Karr (1981,1986) stated that Bluegill exhibited a moderate-intolerance to pollution and habitat degradation. We found that our first site, at Dodridge Rd., had the highest relative abundance of Bluegill compared to the remaining sites (Table 2). We believe our findings are consistent with Karr's (1981, 1986) tolerance classification of Bluegill. This is because we found

that as habitats became more degraded, the relative abundance of Bluegill decreased (Fig. 7). Karr (1981, 1986) also stated that Green Sunfish exhibited a high tolerance to pollution and habitat degradation. We found that as habitats became more degraded, there was a non-significant trend toward the relative abundance of Green Sunfish increasing. Hybrid Sunfish have not been given tolerance classification; however, our findings suggest a tolerance classification for Hybrid Sunfish similar to Green Sunfish. This is because their relative abundance followed a very similar trend of increasing relative abundance with decreasing habitat quality; however, the trend illustrated by Hybrid Sunfish was non-significant as well. Although the relationships between Green Sunfish and habitat quality, and Hybrid Sunfish and habitat quality, were not statistically significant, they were found to illustrate a semi-strong correlation (Fig. 7). The trends that were observed for these two species were associated with correlation coefficients that were less than that of the trend illustrated by Bluegill (Fig. 7). We believe this confirms the tolerance classification of Green Sunfish, because their abundances are suggested to be much less affected by the quality of their habitat, in comparison to Bluegill. Our data also confirms the tolerance classification of Bluegill as moderately-intolerant. We found that the abundances of Bluegill are much more affected by the quality of their habitat. We believe the moderate-intolerance label, given by Karr (1981 and 1986) is accurate, because Bluegill were found to be intolerant to decreasing habitat quality; however, individuals were still found at sites classified as poor-quality, supporting the term moderate in the Bluegill classification. If the relationship between Hybrid Sunfish and habitat quality was significant, our findings would have supported the classification of Hybrid Sunfish as tolerant. For now, their tolerance remains unclassified.

Our findings also support the early work of Werner and Hall (1977) who found a loss of instream cover and an increase in sedimentation have a negative effect on the amount and availability of overall suitable habitat, thus increasing competition and potentially driving hybridization. The study sites with the greatest abundances of Hybrid Sunfish and the least abundances of Bluegill were also the sites with the least amount of instream cover and the greatest amount of siltation and sedimentation. In our search to determine critical habitat quality scores, we were able to determine which habitat variables contributed to the greatest variance in total habitat quality, substrate and instream cover. These variables differed the most between sites, and could therefore possibly be the habitat variables that are driving hybridization between Bluegill and Green Sunfish. Substrate and instream cover were the habitat variables that we initially hypothesized to have the greatest influence on hybridization. Walters et al. (2003) found that as sedimentation increased, fish assemblages became homogenized. In our sites with the greatest sedimentation, this appears to be what is happening. Because of the fertility of Hybrid Sunfish, they are able to backcross and reproduce with “pure-bred” Bluegill and Green Sunfish. This could allow for the homogenization of species into one, Hybrid Sunfish, further decreasing biodiversity in these micro-ecosystems. While our research suggests that sedimentation and instream cover are the two variables with the greatest influence on hybridization between these two species in degraded urban streams, with such a limited dataset we lack the statistical power to make such claims.

## **Conclusions**

The findings of our research align with what is already known about the relationship between habitat degradation and aquatic biodiversity. Sites with the greatest amount of

degradation exhibit the lowest amount of biodiversity, at least in this short section of the Olentangy River and with respect to centrarchids. Our findings also supported our latter hypothesis, stating that a negative relationship between habitat degradation and Bluegill abundances exists. Lower abundances of Bluegill were found in sites with greater habitat degradation. There were also greater abundances of Hybrid Sunfish in the sites with the least quality sediment and the least amount of instream cover. The sediment in these sites was silted over and had high levels of embeddedness and the amount of instream cover was minimal. These findings begin to support our primary hypothesis; however, our findings were not statistically significant, but there were strong trends in the predicted direction. Overall, we were able to determine a general relationship between habitat degradation and the relative abundances of Bluegill, Green Sunfish, and Hybrid Sunfish. That is, as the amount of habitat degradation increased, the relative abundance of Hybrid Sunfish and Green Sunfish increased.; and, as the amount of habitat degradation increased, the relative abundance of Bluegill decreased. We were also able to determine two habitat variables that are likely influential in this relationship, sediment and instream cover, but we were unable to investigate the effect that turbidity has on hybridization. More research and collection data needs to be completed before we can begin to fully understand the inner workings of the effect that aquatic habitat degradation has on hybridization between Bluegill and Green Sunfish.

## **Acknowledgements**

This research was completed under the IACUC protocol number: 2014A00000055. Thank you to the Ohio State University, School of Environment and Natural Resources Honors and Scholars Program, for providing the opportunity to take part in undergraduate research. Also,

thank you to Dr. Suzanne Gray and the OSU Wetlands Research Park for providing the laboratory and equipment needed for sampling. Finally, thank you to my laboratory partners and friends: Jeff Robbins, Makayla McKinney, Erin O'Shaughnessey, and Chelsea Nieman for support and assistance in both the field and laboratory.

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### Tables and Figures

**Table 1:** Qualitative Habitat Evaluation Index (QHEI), its metrics, method of measurement, and scores.

<b>Metric</b>	<b>Method of Measurement</b>	<b>Total Maximum Score</b>
Substrate	Indicate best type, other types, source of origin, quality, and level of embeddedness	20
Instream Cover	Indicate all types and amount of available cover (as a percentage)	20
Channel Morphology	Indicate sinuosity, development, channelization, and stability	20
Bank Erosion and Riparian Zone	Indicate erosion, riparian width, flood plain quality (on both sides of stream)	10
Pool Quality	Indicate maximum depth, channel width, and current velocity	12
Riffle Quality	Indicate depth, run depth, riffle/run substrate, and riffle/run embeddedness	8
Gradient	Indicate gradient (very low – very high)	10
Total QHEI score	Sum the above scores	100

**Table 2:** Total counts and relative abundances of each species caught in the present study.

<b>Species</b>	<b>Site 1</b>	<b>Site 2</b>	<b>Site 3</b>	<b>Site 4</b>
Bluegill	10 (76.92%)	2 (13.33%)	0 (0.00%)	1 (25.00%)
Green Sunfish	2 (15.38%)	6 (40.00%)	7 (58.00%)	1 (25.00%)
Hybrid	1 (7.69%)	5 (33.33%)	5 (42.00%)	2 (50.00%)
Orangespotted Sunfish	0 (0.00%)	2 (13.33%)	0 (0.00%)	0 (0.00%)

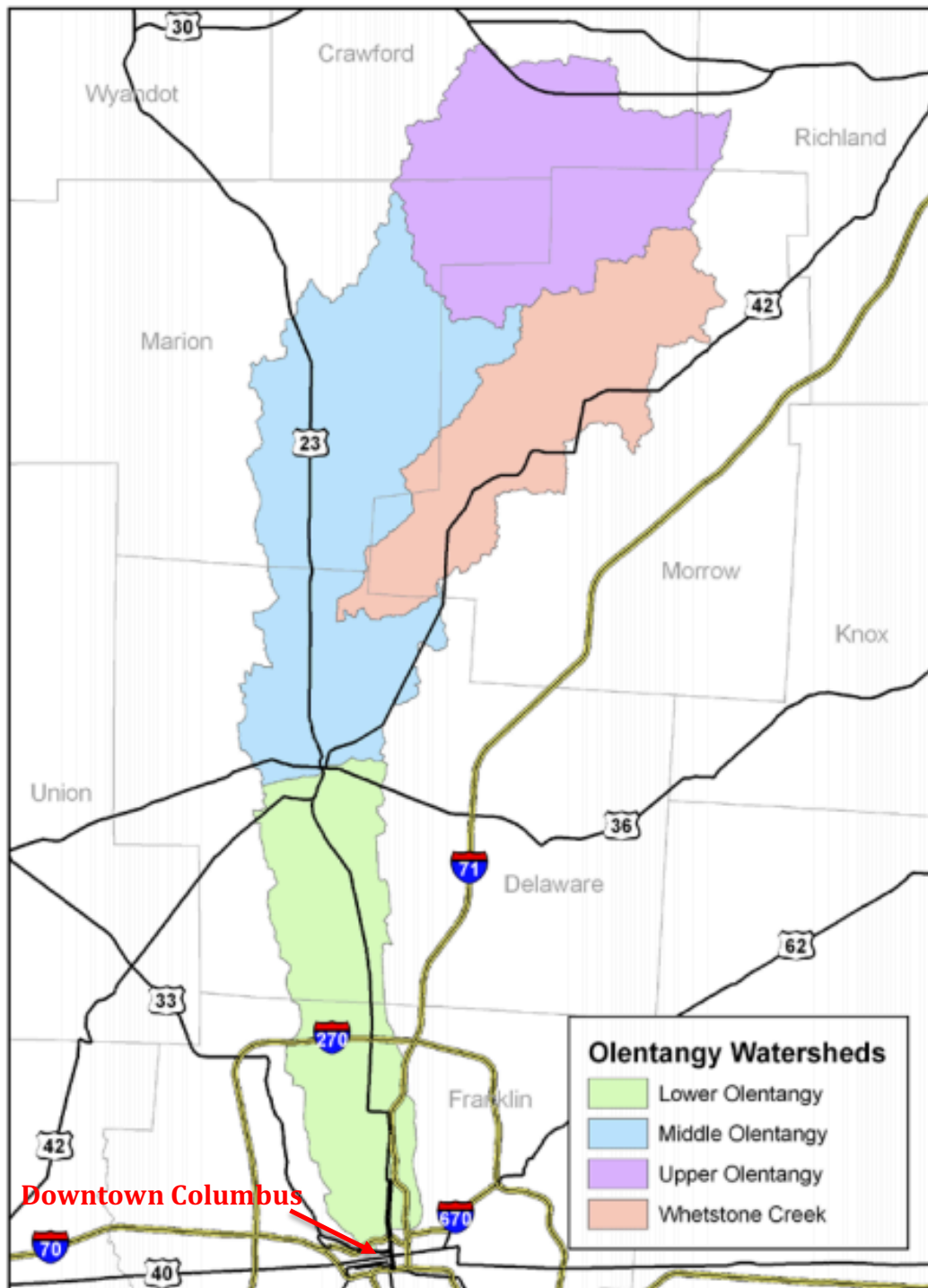
**Table 3:** Turbidity and water quality data from each site.

Site	Average Turbidity (NTU)	Average	Standard Dev	Temp ( C )	D.O. mg/L	Average D.O.	Standard Dev	Conductivity microsiemens/cm	Conductivity millisiemens/cm
1	12.40	6.01	5.56	24.7	7.28	8.01	1.27	461	0.464
1	3.48			21.5	7.27			600	0.633
1	2.18			20.32	9.48			699	0.704
2	5.76	4.78	0.89	21.5	10.68	8.29	2.08	680	0.729
2	3.99			25.4	6.88			503	0.499
2	4.62			22.3	7.31			635	0.696
3	4.27	3.36	1.17	19.7	8.85	7.98	0.85	787	0.793
3	2.03			22.6	7.15			594	0.610
3	3.79			21.6	7.95			700	0.712
4	3.71	6.78	5.83	15.6	9.45	9.00	1.29	609	0.741
4	3.12			21.5	10.02			715	0.766
4	13.51			24.3	7.54			473	0.481

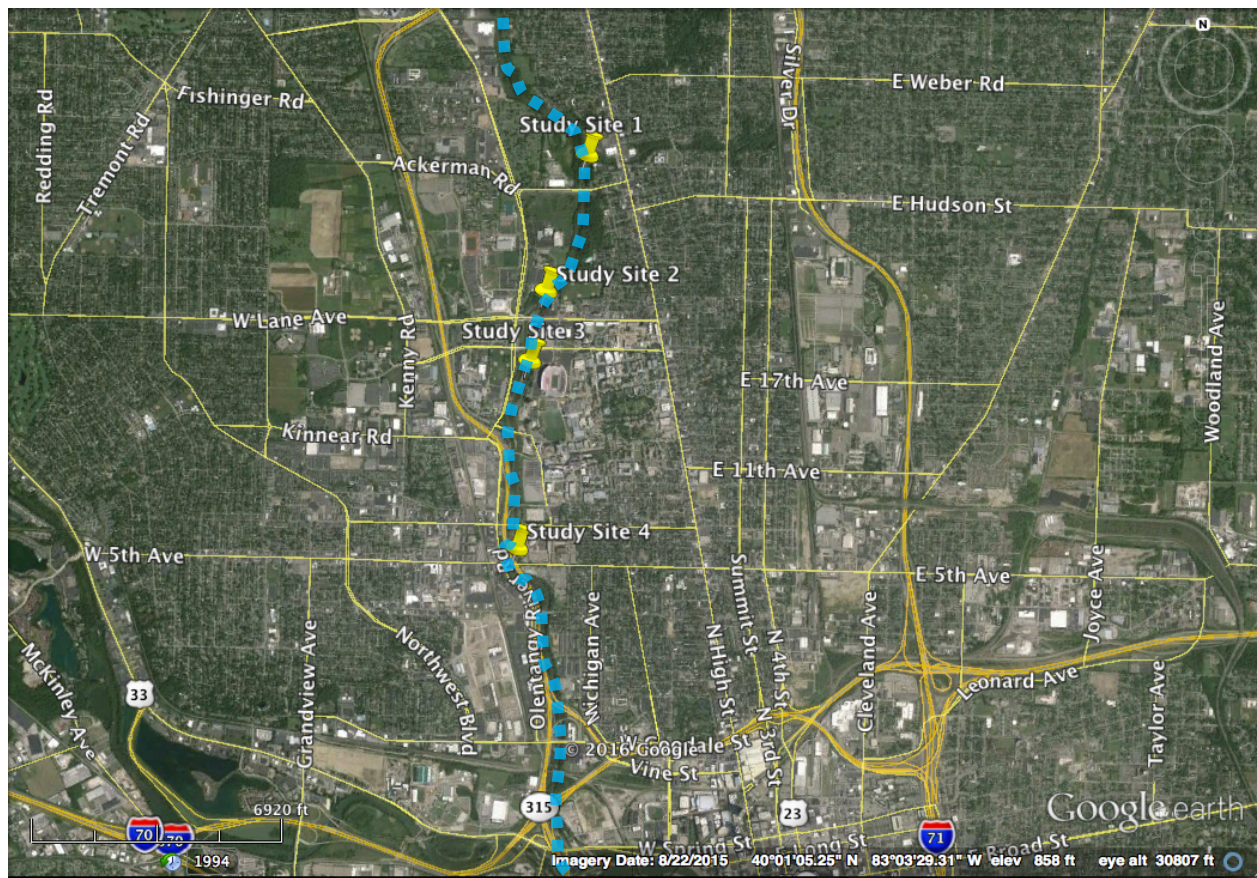
**Table 4:** Qualitative Habitat Evaluation Index (QHEI) scores and total score for each site.

	<b>Score</b>						
<b>Metric</b>	<b>Site 1</b>	<b>Site 2</b>	<b>Site 3</b>	<b>Site 4</b>	<b>Average</b>	<b>Std. Deviation</b>	<b>Coeff. Of Variation</b>
Instream Cover	8	5	4	5	5.5	1.73	0.315
Substrate	11	7	5.5	7	7.63	2.36	0.309
Riffle Quality	7	4	5	4	5	1.41	0.283
Pool Quality	12	10	6	9	9.25	2.50	0.270
Bank Erosion and Riparian Zone	6	4	4	3.5	4.38	1.11	0.253
Channel Morphology	7	8	7	8	7.5	0.577	0.077
Gradient	2	2	2	2	2	-	-
Total	53	40	33.5	38.5	41.3	-	-

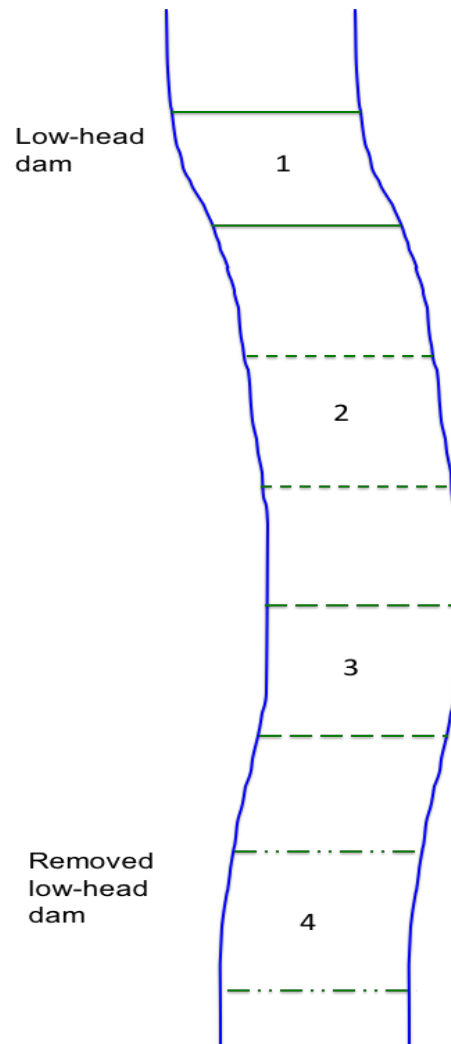




**Figure 1:** Olentangy River Watershed, broken up into four sections, in relation to Columbus, Ohio and Ohio counties (Ohio EPA, 2007).

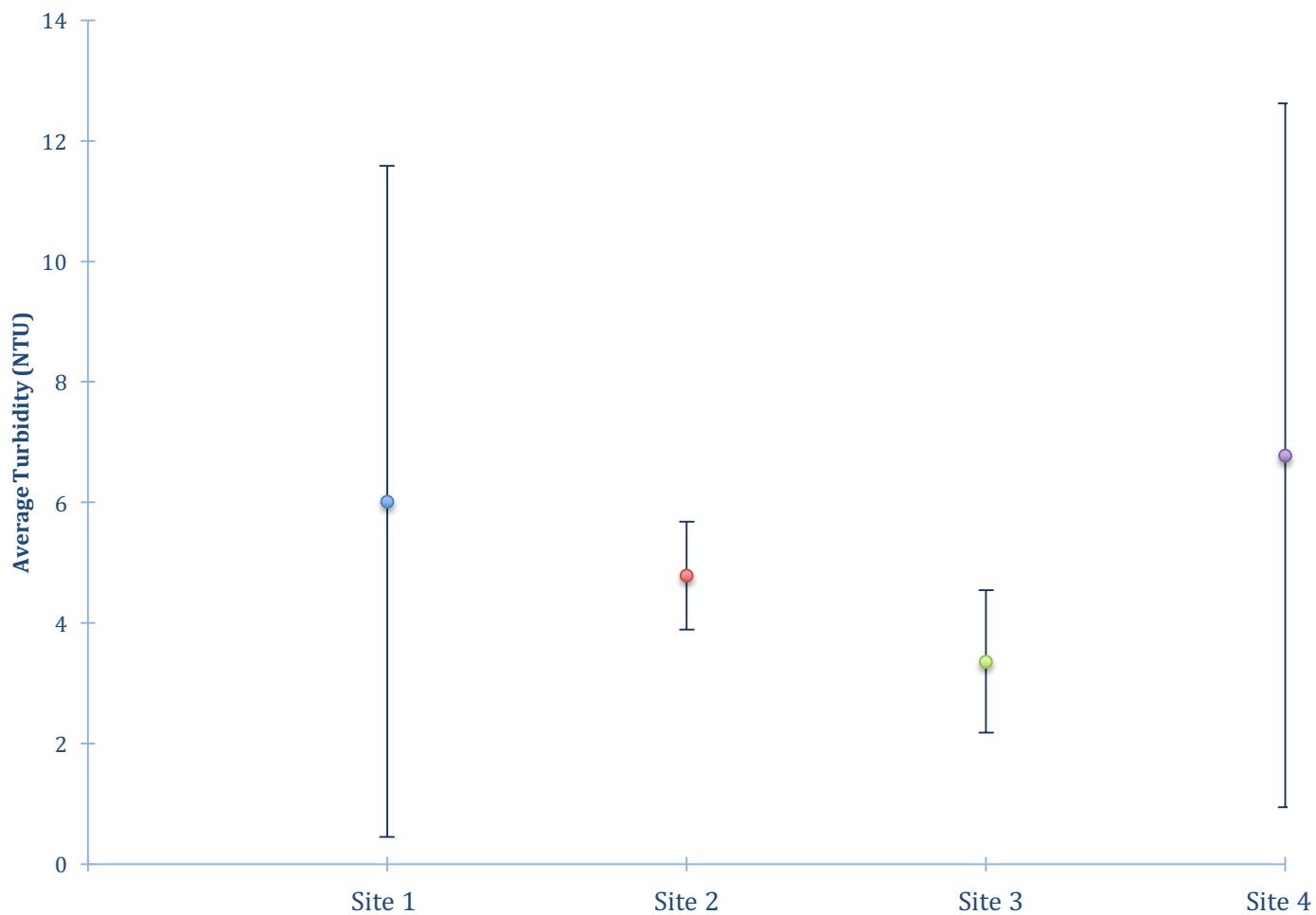


**Figure 2:** Study sites 1-4 illustrated as yellow pins on map of Olentangy River (blue dashed line) and nearby roads/highways (Google Earth, 2016).

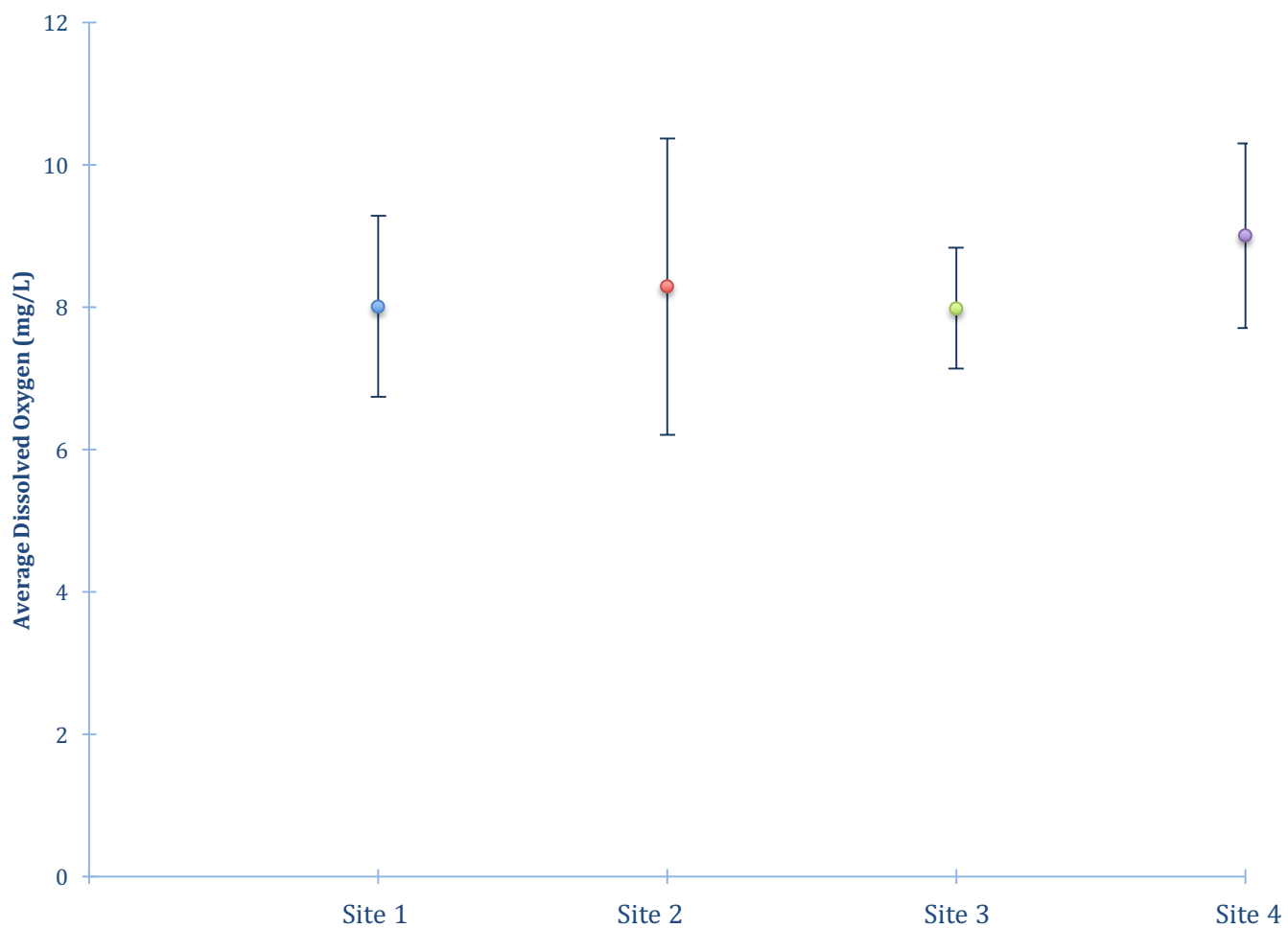


**Figure 3:** Placement and location of study regions (1-4), 300m long, along the Olentangy River.

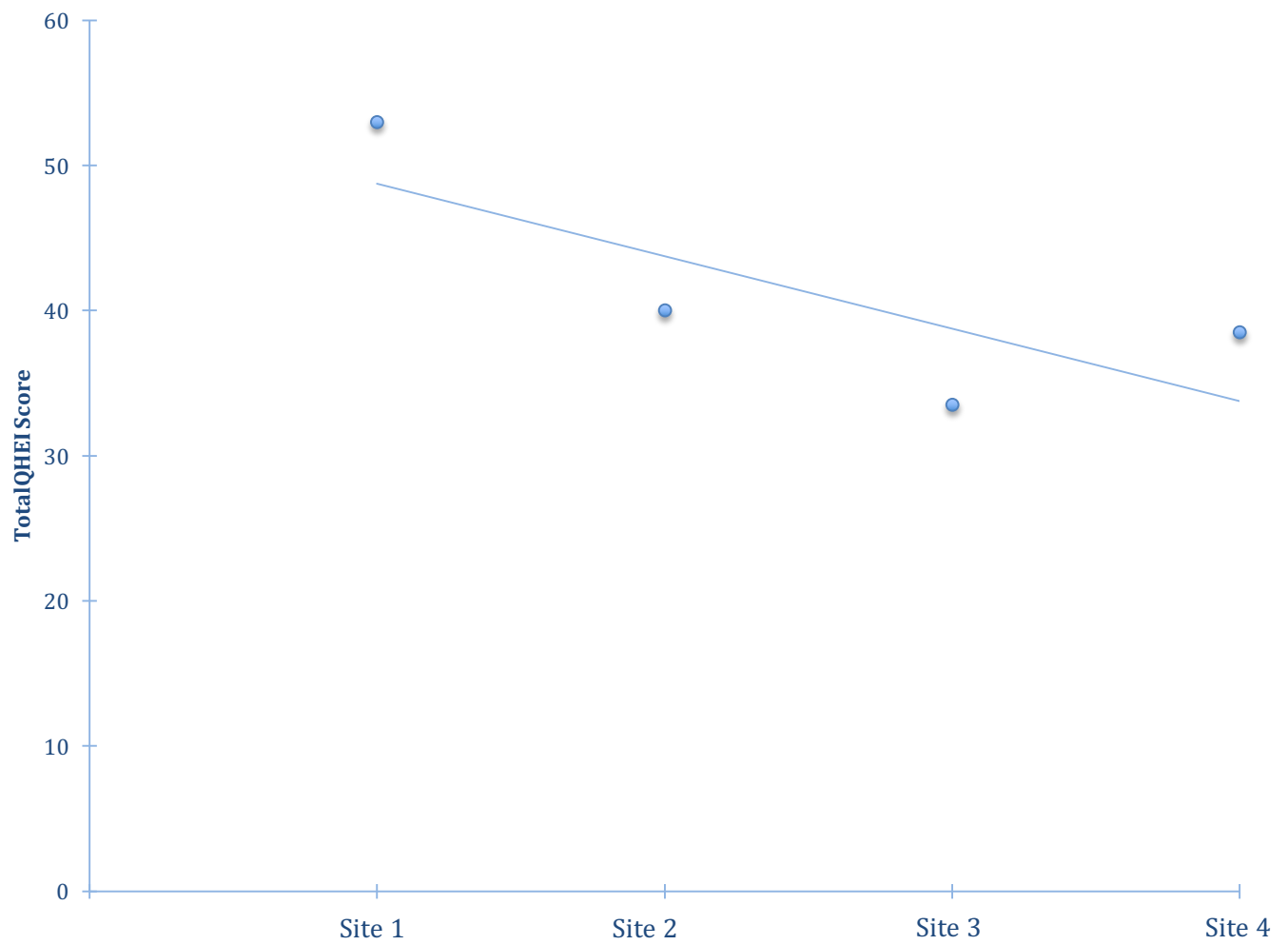
Solid lines depict current or previous dam locations. Low-head dam is directly upstream from Dodridge Road. Removed low-head dam was located on 5<sup>th</sup> Avenue.



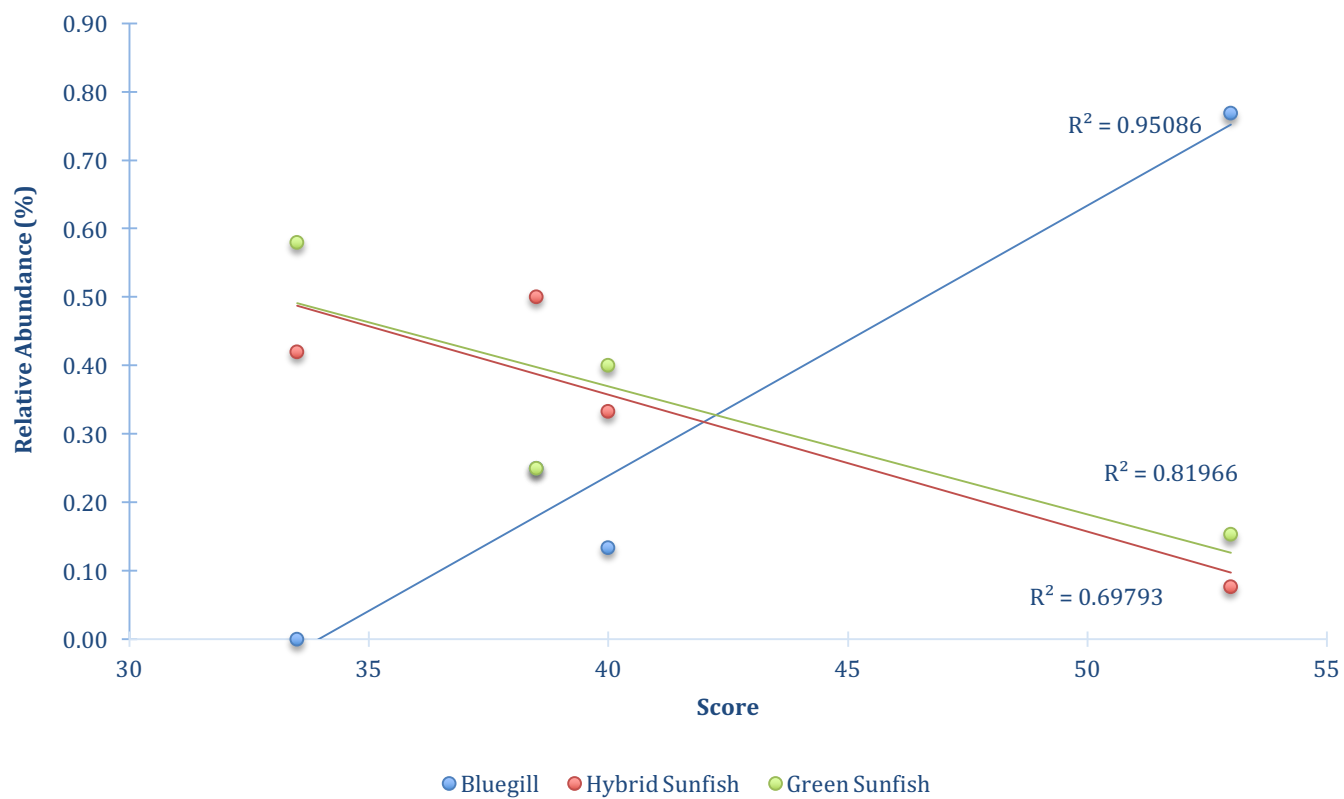
**Figure 4:** Average turbidity (NTU) for each site over the course of the study. Error bars are standard deviations.



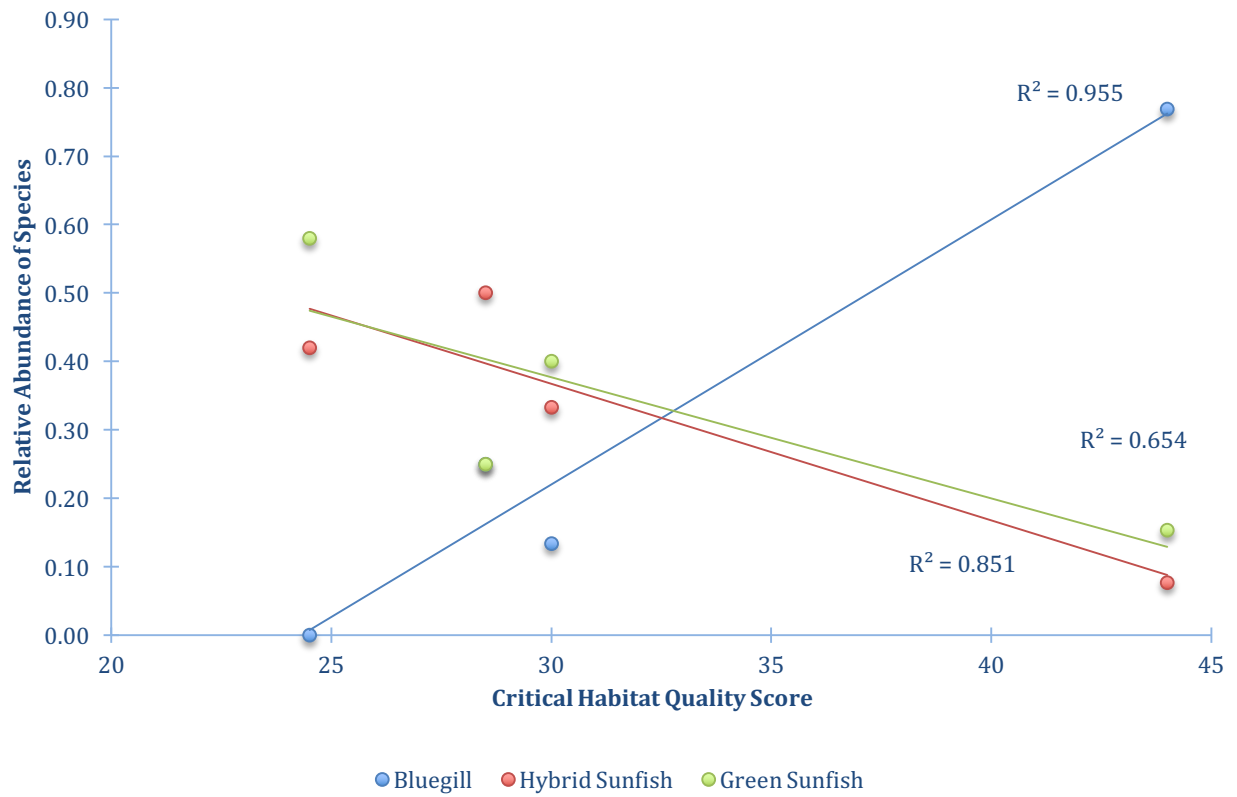
**Figure 5:** Average dissolved oxygen for each site over the course of the study. Error bars are standard deviations.



**Figure 6:** Total QHEI scores for each study site.

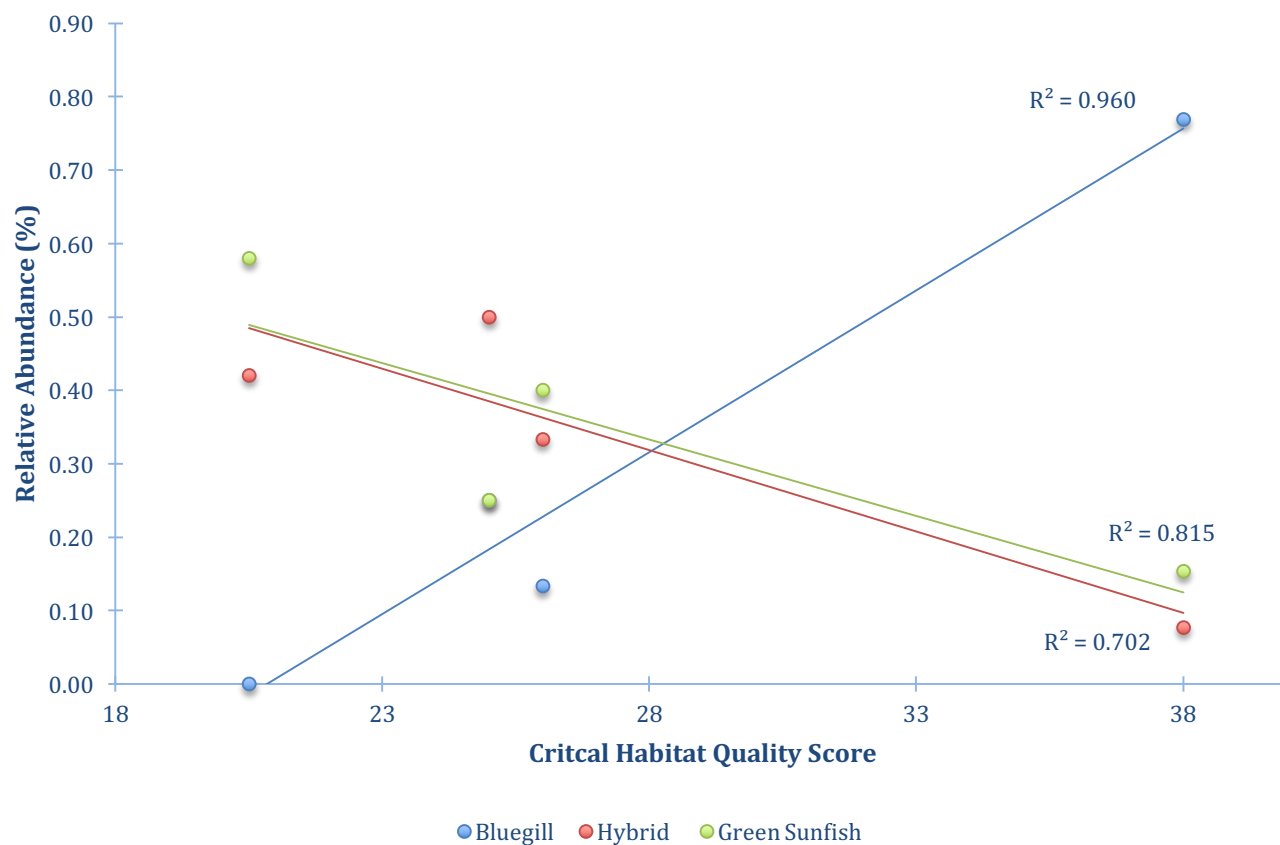


**Figure 7:** Relative abundances of Bluegill, Green Sunfish, and Hybrid Sunfish as a function of total QHEI scores.

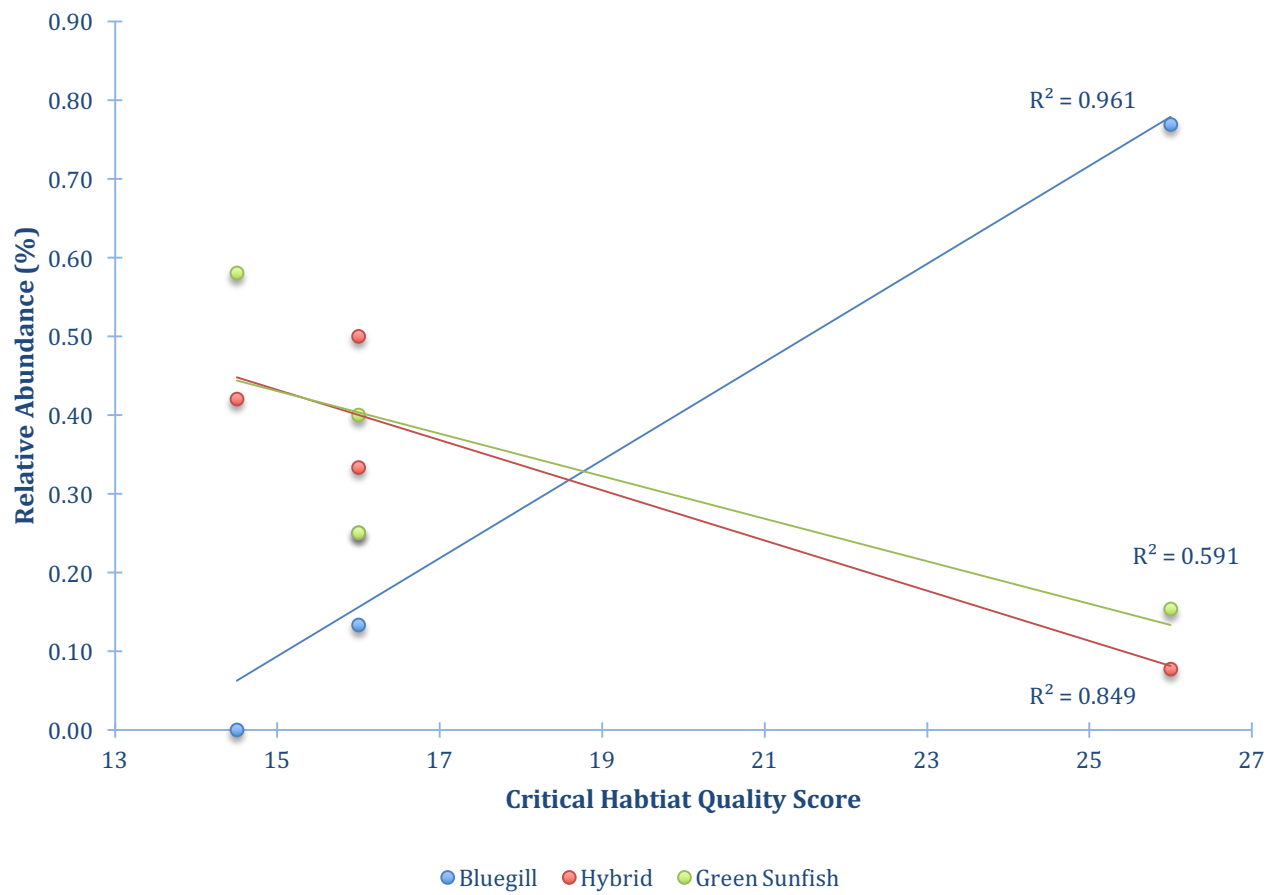


**Figure 8:** Relative abundances of Bluegill, Green Sunfish, and Hybrid Sunfish as a function of critical habitat quality scores (including substrate, instream cover, pool quality, riffle quality, and bank erosion and riparian zone).

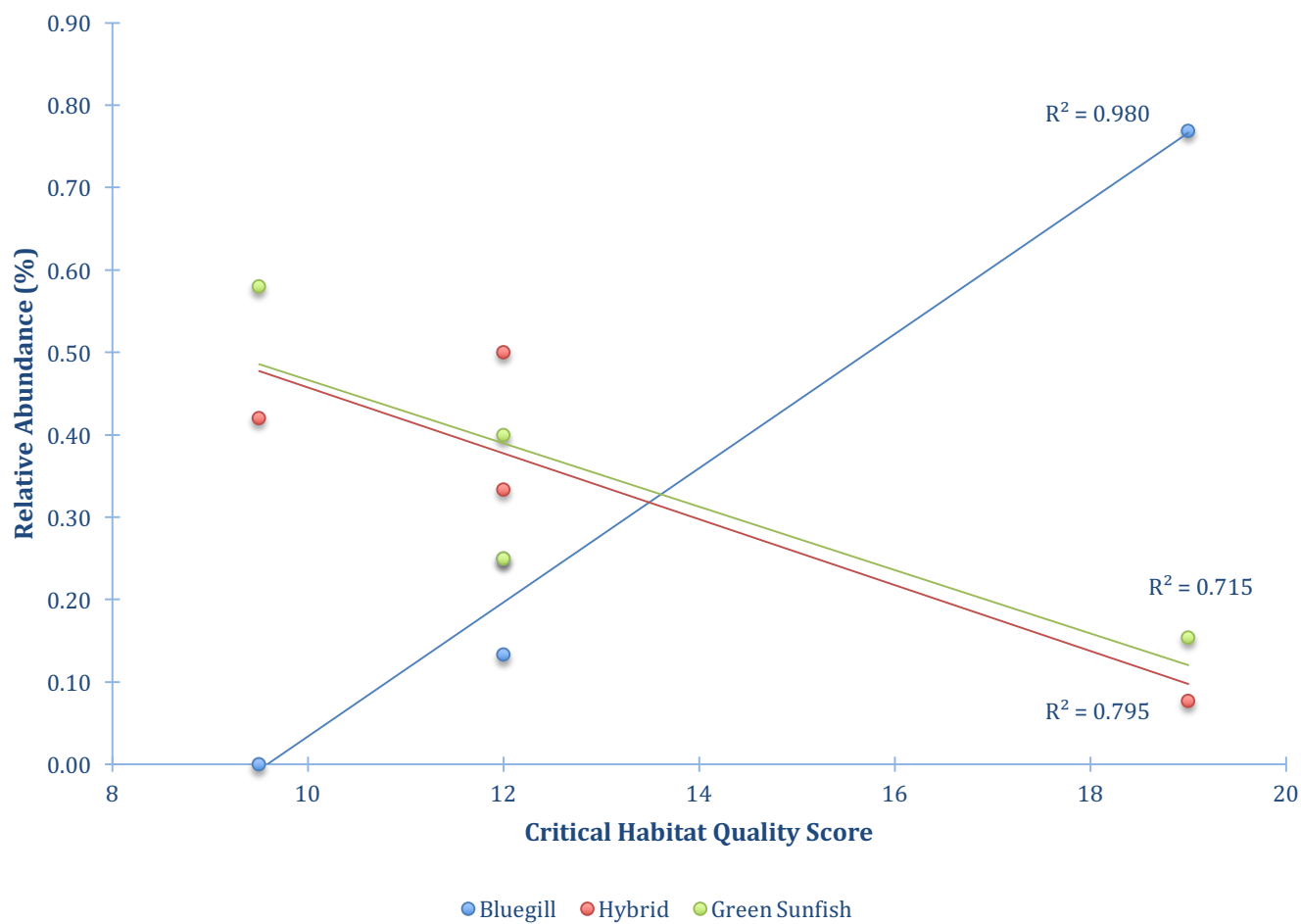




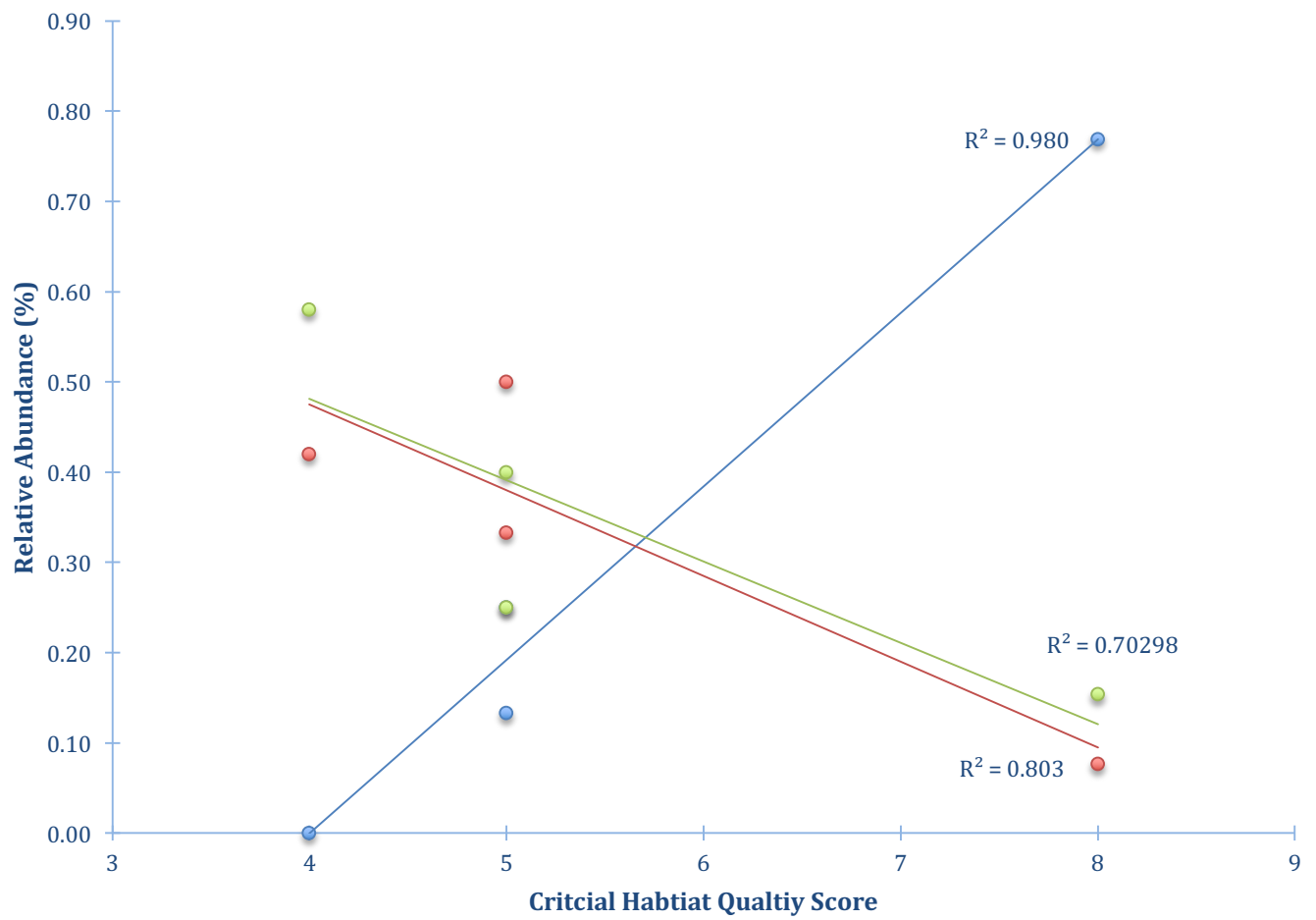
**Figure 9:** Relative abundances of Bluegill, Green Sunfish, and Hybrid Sunfish as a function of critical habitat quality scores (including substrate, instream cover, pool quality, and riffle quality).



**Figure 10:** Relative abundances of Bluegill, Green Sunfish, and Hybrid Sunfish as a function of critical habitat quality scores (including substrate, instream cover, and riffle quality).



**Figure 11:** Relative abundances of Bluegill, Green Sunfish, and Hybrid Sunfish as a function of critical habitat quality scores (including substrate and instream cover).



**Figure 12:** Relative abundances of Bluegill, Green Sunfish, and Hybrid Sunfish as a function of critical habitat quality scores (instream cover score).